

CLAIMS

1. A protein comprising the sequence Seq ID No 1 or Seq ID No 4, or a variant thereof, capable of hydrolysing sphingomyelin.
- 5 2. The protein according to claim 1, wherein the Seq ID No 1 or Seq ID No 4 or variant thereof is capable of hydrolysing sphingomyelin at pH 7.5-9.
3. The protein according to claim 1, wherein the Seq ID No 1 or Seq ID No 4 or variant thereof has >50% of its hydrolysing activity at pH >7.5.
- 10 4. The protein according to claim 1, wherein the variant has at least 80% identity with Seq ID No 1 or Seq ID No 4.
5. A nucleotide sequence encoding the protein according to any of claims 1-4.
- 15 6. The nucleotide sequence according to claim 5, wherein the nucleotide sequence comprises Seq ID No 2 or Seq ID No 5.
7. A recombinant expression and secretion vector comprising a polynucleotide encoding a secretion signal peptide; a DNA sequence which promotes transcription in a host cell located upstream from the polynucleotide encoding the secretion signal peptide; a DNA sequence encoding a protein according to any of claims 1-4 in a translation reading frame with said polynucleotide encoding the secretion signal peptide; and a transcription terminator sequence located downstream from the DNA sequence encoding said protein.
- 20 8. A host cell comprising the recombinant expression system according to claim 7 from which Alk-Smase is expressed.
- 25 9. The host cell according to claim 8, wherein the host cell is a bacteria, a mammalian cell or a yeast cell which in the absence of the recombinant expression system according to claim 7, does not normally produce an Alk-Smase.
- 30 10. A method for isolation of human Alk-Smase protein, the method comprising the steps of
 - xvi) providing a small intestinal or colon content from a human,
 - xvii) homogenising the small intestinal or colon content

xviii) purifying Alk-Smase using DEAE Sephadex chromatography
xix) purifying using Uno anion exchange chromatography,
xx) purifying using hydrophobic chromatography,
thereby isolating the human Alk-Smase protein.

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11. A method for preparation of recombinant Alk-Smase protein capable of hydrolysing sphingomyelin, the method comprising the steps of
ix) providing a host cell according to any of claims 8-9 and a host cell growth medium,
10 x) preparing a host cell culture;
xi) culturing the host cell culture and
xii) harvesting the host cell culture and recovering the human recombinant Alk-Smase.

15 12. The method according to claim 11, wherein the Alk-Smase protein is recovered either from the culture medium, the host cells or after separating the host cells from the culture medium.

20 13. An isolated Alk-Smase protein, comprising the protein according to any of claims 1-4, having an active site with amino acid sequence AFVTMTSPCHFTLVTGKY (Seq ID No 3) or a variant thereof.

25 14. A composition comprising a protein according to any of claims 1-4, or a nucleic acid according to any of claims 5-6, or an isolated Alk-Smase according to any of claims 12-13, and a biocompatible carrier or additive.

30 15. Use of a protein according to any of claims 1-4, or a nucleic acid according to any of claims 5-6, or an isolated Alk-Smase according to any of claims 12-13, for the preparation of a pharmaceutical composition for the treatment of colon cancer.

16. A kit comprising the protein according to any of claims 1-4, or the isolated protein according to claim 13, and a stabiliser.

35 17. The kit according to claim 16, wherein the protein is in a lyophilised form or freeze-dried form.